

What role for AHR activation in IL4I1-mediated immunosuppression ?

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ABSTRACT

The amino-acid catabolizing enzyme Interleukin-4 induced gene 1 (IL4I1) remains poorly characterized despite it is emerging as a pertinent therapeutic target for cancer. IL4I1 is secreted in the synaptic cleft by antigen-presenting cells. It inhibits TCR signaling, modulates naïve T cell differentiation and limits effector T cell proliferation. IL4I1 expression in tumors shapes the tumor microenvironment and impairs the antitumor cytotoxic T cell response, thereby facilitating cancer immune escape. Several mechanisms participate in these effects. Recent data suggest a role of new IL4I1 metabolites in activation of the aryl-hydrocarbon receptor (AHR). Here, we observe that expression of IL4I1 is poorly correlated with that of validated targets of AHR in human cancers. Moreover, dendritic cells do not upregulate AHR target genes in relation with IL4I1 expression *in vivo*. Finally, IL4I1 activity toward tryptophan leading to production of AHR-activating products is very low, and should be negligible when tryptophan-degrading enzymes of higher affinity compete for the substrate. We recently showed that IL4I1 expression by dendritic cells directly regulates immune synapse formation and modulates the repertoire and memory differentiation of responding CD8 T cells after viral infection. Thus, IL4I1 may restrain tumor control through regulating the priming of tumor-specific CD8 T cells, independently of AHR activation.

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Introduction

Amino-acid catabolizing enzymes, responsible for the diminution of T-cell activation, proliferation, and/or function, have gained growing importance in immunology over the last 20 years.¹ Several are now clearly perceived to be central actors of tumor escape from immunosurveillance. Illustrating this interest, indoleamine 2,3-dioxygenase (IDO1) has been proposed as a possible target for the treatment of cancer and small-molecule inhibitors have been tested in preclinical models and Phase I/II trials, with promising results. However, in a Phase III clinical trial in melanoma patients, the absence of any additional clinical benefit of the most advanced molecule, epacadostat, in combination with pembrolizumab has prompted rethinking of this strategy.² Other immunosuppressive enzymes represent possible candidates for chemical inhibition.

Since the first study to identify human IL4I1 as a phenylalanine (Phe) oxidase secreted by antigen-presenting cells (dendritic cells [DC], monocytes, macrophages, and B-cells) in 2007, it has also been demonstrated to inhibit human T-cell proliferation *in vitro*.^{3,4} Both CD8⁺ and CD4⁺ T cells were equally inhibited, but CD45RO⁺ (memory) cells were more sensitive than CD45RA⁺ (naïve) cells. IL4I1 also facilitated the differentiation of FoxP3⁺ regulatory T cells from naïve CD4⁺ T cells.⁵ IL4I1 is produced by the macrophages that infiltrate most human solid tumors and by cancerous cells in the case of certain B-cell lymphoma types.⁶ Recent single cell analyses have also identified IL4I1 expression in tolerogenic

DC subpopulations of the tumor microenvironment, which can express *IDO1*.^{7,8}

The role of IL4I1 has been characterized in mouse models of transplanted and spontaneous melanoma, both in WT and IL4I1 deficient backgrounds, clearly showing that it facilitates tumor growth by inhibiting the antitumor cytotoxic T-cell response and remodeling the tumor immune microenvironment.^{9,10} These observations have been recently extended to a model of chronic lymphocytic leukemia.¹¹ In line with this, clinical correlations have been reported between IL4I1 expression by stromal cells and invasion of the sentinel lymph nodes, a higher melanoma stage, and rapid relapse in human primary cutaneous melanomas, in which IL4I1 expression was analyzed by immunohistochemistry.¹² Most interestingly, zones in which IL4I1 expression was concentrated were depleted of cytotoxic CD8⁺ T cells and enriched with regulatory FoxP3⁺ T cells. Moreover, *IL4I1* was overexpressed in melanoma patients with progressive disease under treatment with the anti-PD-1 antibody nivolumab, suggesting a relation between IL4I1 expression and resistance to immune-checkpoint blockade.¹¹

We initially proposed IL4I1 as a prognostic biomarker and therapeutic target in cancer in 2009.⁶ Transcriptomic data from several groups also indicated that IL4I1 is associated with poor outcome in certain human carcinomas (breast, renal, and colon carcinoma and glioma) (13 and reviewed in 14).^{13,14} Sadik *et al* recently showed IL4I1 expression in tumor cells of low-grade gliomas and glioblastomas and observed correlation with

diminished patient survival.¹¹ Glioblastoma is particular in that it is suspected to be derived from neural stem cells¹⁵ and isoform 2 of IL4I1 is expressed in several neural cell types,¹⁶ with a still undefined function that may involve control of the level of neurotransmitter amines.^{17,18} In the same work, they demonstrate for the first time that IL4I1 promotes glioblastoma motility *in vitro*, an effect that may be highly relevant for metastatic processes. Finally, their work brings new insights by proposing that the immunosuppressive effect of IL4I1 in tumors is mediated by activation of the ligand-activated transcription factor aryl hydrocarbon receptor (AHR). AHR is widely distributed in tissues and displays very diverse activities, depending on the cellular and molecular context. Its role in cancer is complex and incompletely deciphered, involving both pro and anti-tumorigenic effects that affect directly tumor cells or the tumor microenvironment.¹⁹ Here, we examine the hypothesis of IL4I1-mediated AHR activation and propose a model of IL4I1 action during cancer development.

Material and methods

Transcriptomic analysis of human primary tumors and mouse DCs

Data from human primary tumors: breast, colon, and ovarian cancer, melanoma, mesothelioma, and glioblastoma were retrieved from The Cancer Genome Atlas (TCGA) using the Xena platform. Among genes used in the Sadik *et al.* study,¹¹ we defined 45 experimentally-confirmed AHR target genes according to the Harmonizome platform,²⁰ which report genes identified by Lo and Mathews.²¹ Spearman's rho were calculated using GraphPad Prism 8.

Genes modulated by 2,3,7,8-tétrachlorodibenzo-*p*-dioxine (TCDD) in human cells (hepatocytes, CD34⁺ hematopoietic

stem cells, MCF7 and HL60 cell lines) were retrieved from GSE7765, GSE14553, GSE16160, GSE24193, GSE46874, GSE122518 (NCBI GEO site), based on metaanalysis by Oshchepkova *et al.*²²

DCs were obtained by negative cell sorting from spleens of WT and IL4I1^{-/-} mice at steady state and at 24 h of infection with lymphocytic choriomeningitis virus (LMCV) strain WE2.2, as described in Puiffe *et al.*²³ Mouse experiments were approved by the local Ethical Committee for Animal Experimentation (Cometh Anses/ENVA/UPEC) and the French Research ministry under the number 05338.03.

Total RNA was isolated from purified DCs using RLT buffer and RNeasy columns (Qiagen). Libraries of polyA mRNA were generated using the TruSeq[®] Stranded mRNA Library Prep kit (Illumina) with double indexing using TruSeq RNA UD Illumina Indexes. RNA was reverse transcribed using SuperScript[™] II (Invitrogen). Next-generation sequencing was performed by 75 bp single reading with the NextSeq 500/550 High Output Kit v2.5 (75 Cycles) on a NextSeq 500 analyzer (all from Illumina).

The twelve samples (40,675,782 ± 2,630,770 reads/sample) were quality-checked using the software FastQC (version 0.11.8). We checked that rRNA depletion had the expected quality (less than 2.32 ± 0.88 of total RNA, no prokaryotic contamination) and that more than 93% of the reads mapped to the mouse genome (GRCm38) using SortMeRNA (version 2.1b), FastQScreen (version 0.13) and Kraken2 (version 2.0.9/default database). Trimmomatic (version 0.39) was used to filter reads using a quality 20 (sliding window of 5 reads) and minimal length of 50pb, which led to more than 96% of surviving reads. Filtered reads (39,151,723 ± 2,545,395) were aligned to the mouse genome (GRCm38) using STAR (version 2.6.1d). The mapping of the reads for the different regions of the genome and the level of gene expression was calculated using RSEM (version 1.3.2). The level of gene expression was normalized in CPM (counts

Table 1. Genes of the AHR signature in Sadik *et al.* (n = 166). Forty-five genes identified as AHR targets by CHIP-Seq are in bold.²¹ Blue and red boxes indicate genes that are upregulated and downregulated, respectively, by TCDD in GSE7765, GSE14553, GSE16160, GSE24193, GSE46874, GSE122518. HLA-DRB4 is upregulated in CD34⁺ cells, but downregulated in hepatocytes. The most robustly AHR-dependent genes in this list are *AHRR*, *CYP1A1*, *CYP1B1*, *NFE2L2*, *SLC7A5*, *TIPARP* (all upregulated) according to the metaanalysis of Oshchepkova *et al.*²²

ABCC4	C21orf33	CYP3A4	FOXQ1	IL1B	MSI2	PCK2	SERPINE1	TNFSF9
ABCG2	CARD11	DKK3	FPR2	IL1R2	MYC	PDE2A	SES2	TXNRD1
ACTA2	CAV1	DLX3	GATA3	IL2	NANOG	PDS5B	SH3KBP1	UGT1A6
ADM	CCL5	DUOX2	GFI1	IL6	NCOA2	PER1	SLC10A1	VAV3
AHR	CCND1	EBF1	GHR	INSIG1	NCOR2	PHGDH	SLC3A2	XDH
AHRR	CD36	EDN1	GNA13	IRF8	NDRG1	PIWIL1	SLC7A5	ZIC3
ALDH3A1	CD3E	EGFR	GSTA2	JAG1	NEDD9	PIWIL2	SMAD3	
AMIGO2	CD8A	EGR1	GSTM1	JUP	NFE2L2	PLA2G4A	SMAD7	
AQP3	CDK4	EPGN	HES1	KDM1A	NOS1	PNPLA7	SOCS2	
AREG	CDKN1A	EREG	HIF1A	KIAA1549	NOS3	POLK	SORL1	
ARG2	CFTR	ESR1	HLA-DRB4	KIT	NPTX1	PPARGC1A	SOS1	
ATP12A	CRH	F3	HMOX1	KMO	NQO1	PRDM1	SPRR2D	
ATP5IF1	CXCL2	FAS	HSD17B4	LEPR	NR1H3	PTGS2	STC2	
ATP5ME	CYBB	FAT1	HSPB2	LHCGR	NR1H4	RARA	TFF1	
ATP6AP2	CYP19A1	FBXO32	ID1	LIFR	NR3C1	REL	TGFBI	
BCL11B	CYP1A1	FGFR2	ID2	LPL	NRIP1	RFC3	TGM1	
BCL2	CYP1A2	FIG4	IFNG	LTBP1	NSDHL	RSPO3	TH	
BCL6	CYP1B1	FLG	IGF2	LYN	OVOL1	SCARB1	THBS1	
BLNK	CYP2B6	FOS	IGFBP1	MID1	PAX5	SCIN	TIPARP	
BRCA1	CYP2E1	FOXA1	IKZF3	MMP1	PCK1	SERPINB2	TJP1	

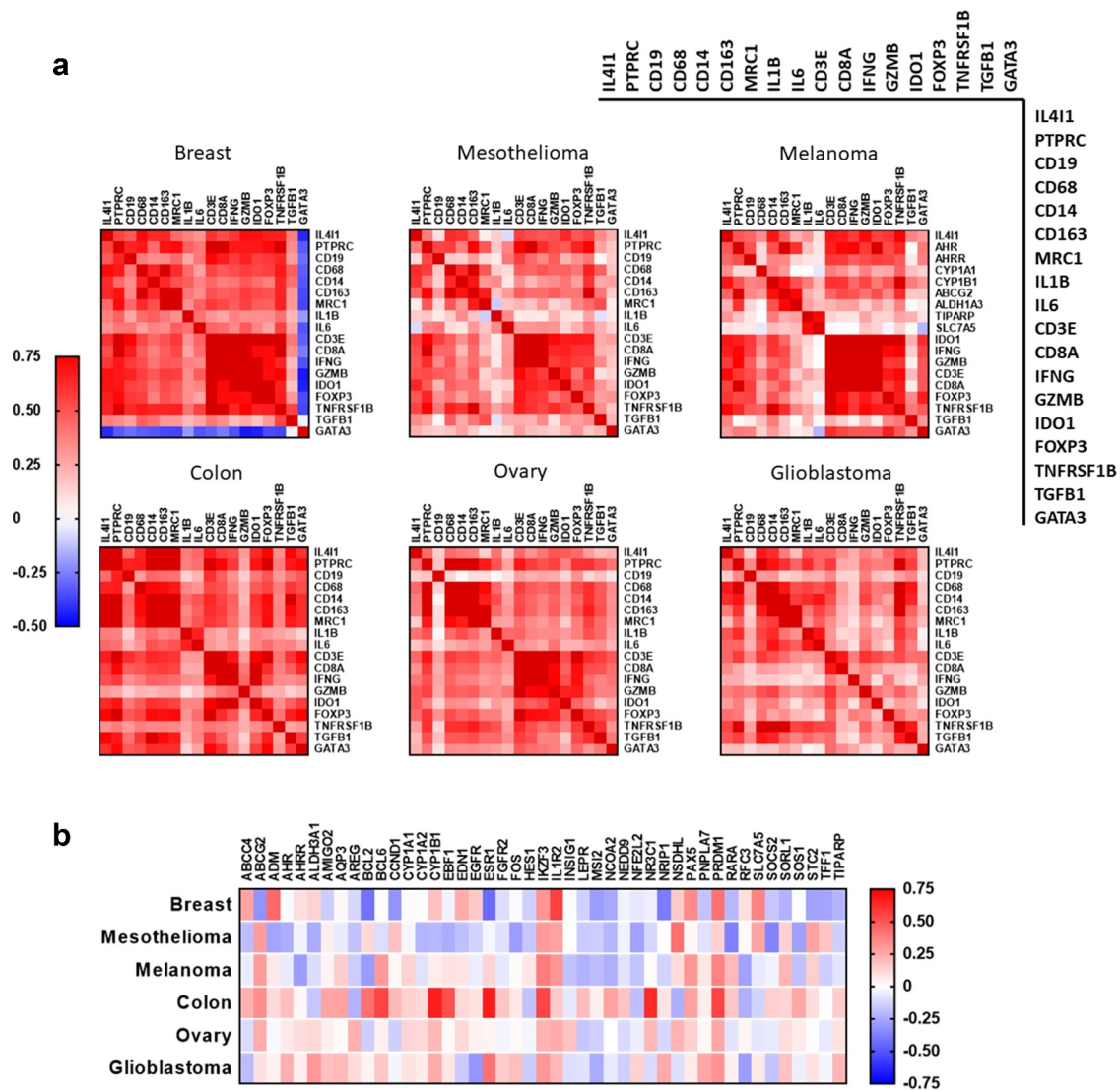


Figure 1. Correlation between the expression of *IL411*, immune-response genes and AHR-regulated genes in primary tumors. (a) Correlation between *IL411* and *PTPRC* (coding the panleukocyte marker CD45), *CD19* (B cells), *CD68*, *CD14*, *CD163*, *MRC1*, *IL1B*, *IL6* (macrophages and inflammation), *CD3E*, *CD8A*, *IFNG*, *GZMB*, *IDO1*, *FOXP3*, *TGFB1*, *TNFRSF1B* and *GATA3* (T-cell infiltration, Th1 response, regulatory T-cells, Th2 cells). (b) Correlation between *IL411* and AHR-dependent genes. Each line shows results for one of the six primary cancer types analyzed. Values above 0.5 are considered highly significant correlations.

per million). A heatmap was generated using GraphPad Prism 8 for the selected AHR-dependent genes and *IL411*, showing the mean CPM from three mice per condition.

IL411 enzymatic activity

Phe and Trp were dissolved in 0.1 N HCl and a 100 ng/ml solution of human recombinant *IL411* (R&D) was used. Hydrogen peroxide production from *IL411* activity was measured at 30 minutes according to Carbonnelle-Puscian *et al.*⁶ with serial dilutions (1250 to 78 μ M) of each amino acid. The K_m and V_{max} were calculated using GraphPad Prism 8. *IL411* activity was also measured on the inducible recombinant HEK cell line expressing *IL411* (described in Boulland *et al.*)³ Lysates in Phosphate Buffer Saline containing protease inhibitors (Complete mini, Roche, Meylan, France) were obtained by 3 freeze-thaw cycles. The enzymatic test was performed as described above, on 40 μ L cell lysates corresponding to

5×10^5 cells. Activity was calculated as pmol H_2O_2 produced per min and per mL.

Results and discussion

Relation between *IL411* expression and activation of the transcription factor AHR

T-cell inhibition by *IL411* has been mainly attributed to its enzymatic activity via the metabolic consequences of Phe consumption and the effect of hydrogen peroxide,^{3,4} although inhibition of early TCR signaling by *IL411* does not involve amino acid deficiency, nor the presence of Phe catabolites.²⁴ Sadik *et al.* establish a correlation between the expression of *IL411* and of the pan-tissue AHR signature they generated. We examined the list of genes used for this AHR signature¹¹ and were puzzled by the fact that it contains several genes, of which the expression may reflect the immune infiltrate of the tumor, in particular, *IFNG*, *CD3E*, and *CD8A* (Table 1). Indeed, the

expression of *IL4I1* in primary tumors from TCGA correlates very strongly with that of T-cell related genes, as well as others, such as *IDO1*, which is indicative of the activation of a Th1 and cytotoxic T-cell response in the tumor bed ($CD3E = 0.57 \pm 0.11$, $CD8A = 0.49 \pm 0.13$, $IFNG = 0.47 \pm 0.13$, $IDO1 = 0.55 \pm 0.15$; mean $\rho \pm SD$ on the six tumor types analyzed; Figure 1(a)). This is in accordance with previous results in human and mouse melanomas^{10,12} and with the known regulation of *IL4I1* expression by interferons.⁴ This is also in line with the reanalysis by Sadik *et al* of data from Riaz *et al*,²⁵ indicating concomitant induction of *IL4I1* and *IDO1* in patients with advanced melanoma treated with anti-PD-1. This induction may result from restored local production of $IFN\gamma$ by reinvigorated T cells. As *IL4I1* is produced by tumor-associated macrophages in most solid tumors,⁶ we also observed a strong correlation between *IL4I1* and various genes attesting the presence of macrophages in the tumor bed ($CD68 = 0.57 \pm 0.15$; $CD14 = 0.63 \pm 0.15$; $CD163 = 0.51 \pm 0.17$; mean $\rho \pm SD$ on the six tumor types analyzed).

A comparison of the AHR signature reported by Sadik *et al.* to validated AHR targets identified by CHIP-seq in the study of Lo and Matthews,²¹ retrieved 45 genes common to both lists, which did not include *IFNG*, *CD3E*, or *CD8A*. We analyzed these 45 genes for correlations with *IL4I1* expression in primary tumors from the TCGA database using the Xena platform²⁶ (Figure 1(b)). With few exceptions, Spearman's ρ was consistently inferior to 0.25; depending on tumor type, 1 to 15 genes had $\rho > 0.25$ and 0 to 9 had $\rho < -0.25$ (total, 1 to 15 genes modulated). Colon carcinoma displayed the highest correlation between *IL4I1* and AHR-regulated genes (15 genes upregulated with $\rho > 0.25$, 7 with $\rho > 0.5$, none downregulated with $\rho < -0.25$). Three of these genes (*CYP1B1*, *ESR1*, *ALDH3A1*) are known to be upregulated by TCDD *in vitro* (Table 1). However, in colon cancer, the diet and/or the microbiota can provide numerous AHR ligands.

To confirm these observations on murine cells, we infected WT and *IL4I1*-deficient (*IL4I1*^{-/-}) mice with LCMV and sorted splenic DCs both at steady state and after infection (Figure 2). Transcriptomic analysis did not show any relationship between *IL4I1* expression and activation of the 45 AHR target genes. Despite vigorous induction of *IL4I1* expression in response to LCMV, AHR-related genes were not significantly upregulated compared to the steady state. In addition, these genes were similarly expressed in DCs from both mouse strains. Thus, the relationship between *IL4I1* expression and induction of the AHR pathway may be only indirect, implying that targeting of the AHR pathway and *IL4I1* for cancer treatment may be independent, and, potentially synergistic.

IL4I1 degradation of tryptophan (Trp)

Sadik *et al.* demonstrate that *IL4I1* degrades the essential amino acid Trp into indole-3-pyruvate (I3P), which is immediately converted *in vitro* to kynurenic acid (KynA) and other derivatives. I3P, KynA, and indole-3-aldehyde all activate AHR.¹¹ A study contemporaneously published by Zhang *et al.* also provides arguments for I3P production by *IL4I1*

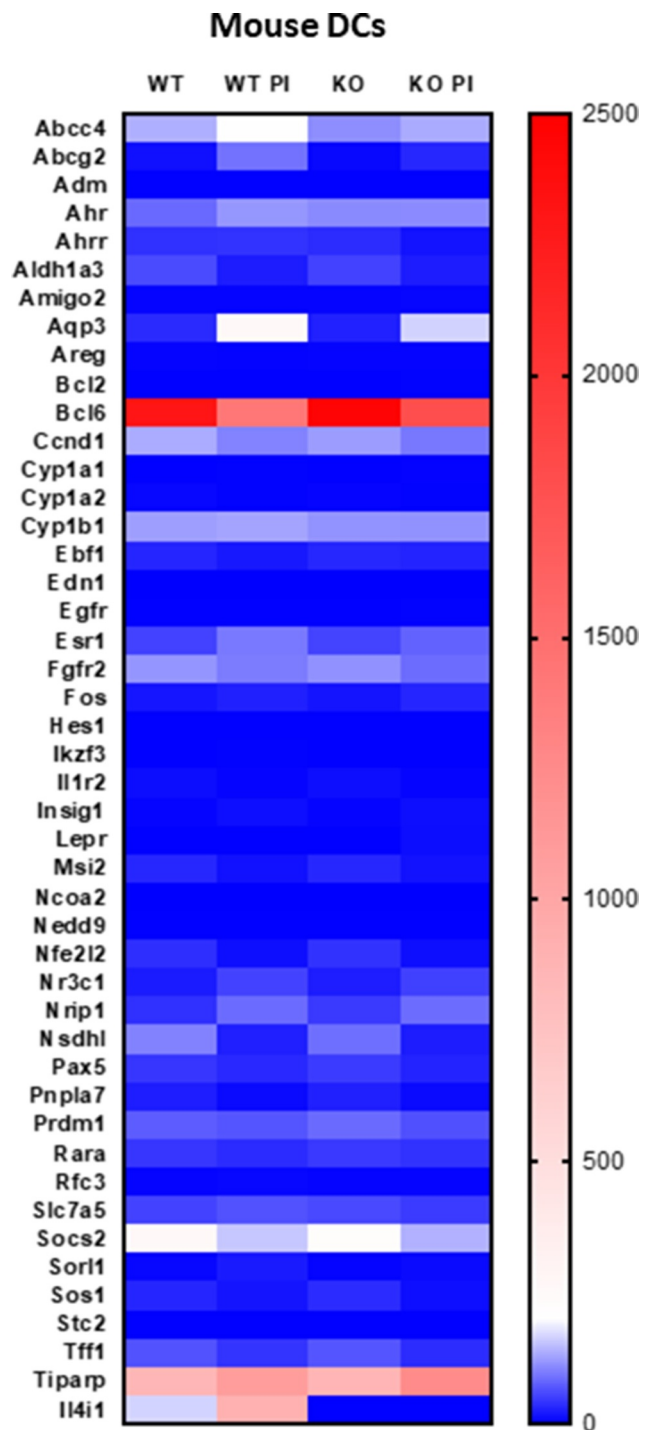


Figure 2. Transcriptomic analysis of purified DCs from WT and *IL4I1*^{-/-} mice at steady state and after 24 h of LCMV infection. The heat map shows counts per million for the selected AHR-dependent genes and *IL4I1* (mean from three mice per group). A previous analysis of the cDNA library at steady state has been published in Puiffe *et al.*²³ PI: post-infection; KO: *IL4I1*^{-/-}.

activity.²⁷ However, *IL4I1* has a strong preference toward Phe. A greater than five-fold higher Phe-degrading than Trp-degrading activity was initially observed using a relatively insensitive colorimetric test.³ Indeed, although measurement of the K_m using an optimized technique shows similar values for the two amino acids, the V_{max} of *IL4I1* toward Phe is almost 10-fold higher than that toward Trp (Figure 3(a)). We validated these data on human cell lysates obtained from

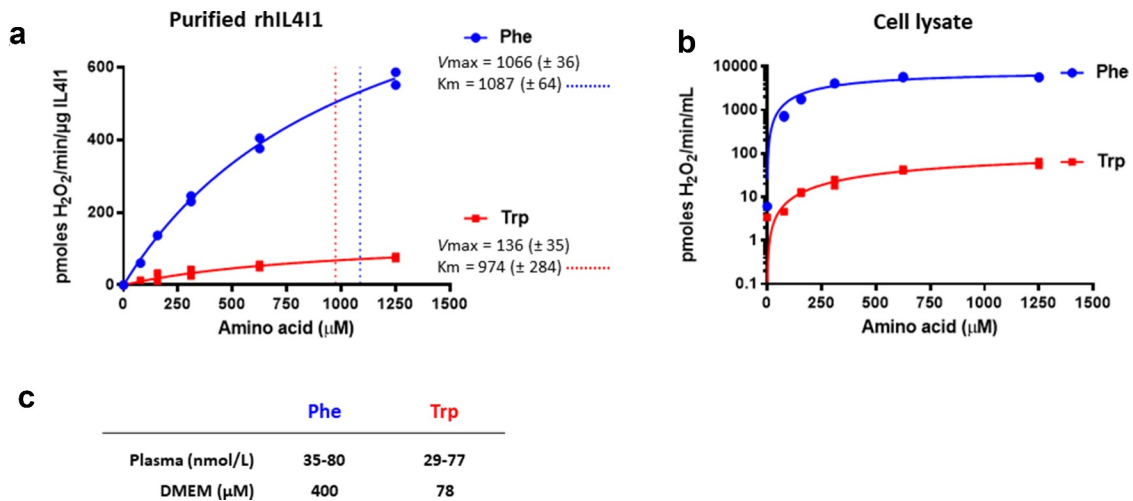


Figure 3. IL4I1 activity toward phenylalanine (Phe) and tryptophan (Trp). (a) Hydrogen peroxide production from the activity of recombinant human IL4I1 was measured in two independent experiments performed in duplicate. Values for K_m and V_{max} (± SD) were determined. The blue and red dotted lines indicate K_m for Phe and Trp, respectively. (b) IL4I1 activity was measured as in A on cell lysates from recombinant HEK cells expressing human IL4I1 (one experiment performed in duplicate). (c) Concentration of Phe and Trp in human plasma (reference values from the Mayo clinic) and in DMEM.

recombinant HEK cells expressing IL4I1 (Figure 3(b)). The difference between Phe and Trp catabolism was even greater than with the purified enzyme. This might be due to different glycosylation, as commercial IL4I1 is produced in Chinese hamster ovary cells. Moreover, in the human body, the concentration of Phe is equal to or slightly higher than that of Trp and the concentration of Phe is substantially higher in classical culture media than that of Trp (Figure 3(c)). Thus, the *in vitro* and *in vivo* catabolism of Trp by IL4I1 should be less than that of Phe and the production of phenylpyruvate should overtake that of I3P. This also suggests that IDO1, whose affinity for the Trp substrate is around 50 folds higher than IL4I1 (Trp K_m~20 μM for IDO²⁸ versus 974 μM for IL4I1) and which is often expressed concomitantly with IL4I1, as stated above, may degrade Trp more efficiently than IL4I1. In accordance, a metabolomic study demonstrates a significant increase in phenylpyruvate and phenyllactate in ovarian cancer in comparison to normal ovary, but does not detect variations in indole compounds.²⁹ Another indirect argument may come from a study in an orthotopic model of mouse pancreatic cancer. In this model, tumor regression after treatment with metformin and torin 2 (mTORC1 and mTORC2 inhibitors) was associated with a 4-fold increase of plasmatic phenylalanine.³⁰ Finally, the use of a specific IL4I1 inhibitor in mouse with B16 melanoma led to significant decrease of phenylpyruvate levels in the tumors.³¹

The I3P produced by IL4I1 in the presence of H₂O₂ (which is a byproduct of the enzymatic reaction) would spontaneously be degraded to KynA, the main mediator of AHR activation.¹¹ However, KynA could be produced independently of IL4I1 activity and experiments showing AHR activation *in vitro* required largely supraphysiological concentrations of the products (3.13 to 40 μM I3P) and Trp consumption required five days of culture to be measured.

Overall, these observations raise questions about the *in vivo* relevance of Trp degradation by IL4I1, in particular, in comparison to the effect of IDO1 on the same substrate. In any case,

treatment with IL4I1 inhibitors should block the enzyme, regardless of the amino-acid substrate, Phe, Trp, or even arginine, which has also been demonstrated to be a minor IL4I1 substrate.¹⁴

Implications for cancer of IL4I1 modulation of CD8 T cell priming

We recently dissected the effect of IL4I1 during the priming of naïve CD8 T cells using a model of acute LCMV infection in WT and IL4I1^{-/-} mice.²³ This work revealed unsuspected aspects of IL4I1 action. Somewhat counterintuitively, the effector CD8 T cell response was stronger in WT (IL4I1⁺) mice than in IL4I1^{-/-} mice. Indeed, the genetic inactivation of *IL4I1* was associated with delayed and slightly diminished expansion of functional short lived-effector CD8 T cells, but enhanced memory T cell differentiation. These observations were not related to intrinsic differences of CD8 T cells between WT and IL4I1^{-/-} mice, but resulted from modulation of immune synapse formation and early activation events by IL4I1-expressing DCs. Indeed, IL4I1 enhanced the T cell activation threshold, thereby favoring the priming of high-affinity clones, restriction of the response to the most immunodominant peptides and rapid acquisition of effector differentiation.

These effects, which are unrelated to AHR activation (Figure 2), are relevant for the T-cell response against cancer. Indeed, IL4I1 expression not only inhibits the proliferation and function of anti-tumor effector T cells in the tumor microenvironment, as previously shown, but its presence in secondary lymphoid organs may restrain the repertoire of primed anti-tumor CD8 T-cells, limiting control of mutant tumor sub-clones. Moreover, as IL4I1 is associated with diminished differentiation of memory CD8 T cells, it may limit the capacity to durably regenerate anti-tumor effector cells. Thus, inhibition of IL4I1 should both improve the antitumor effector response and allow long-lasting revitalized immunity against cancer.

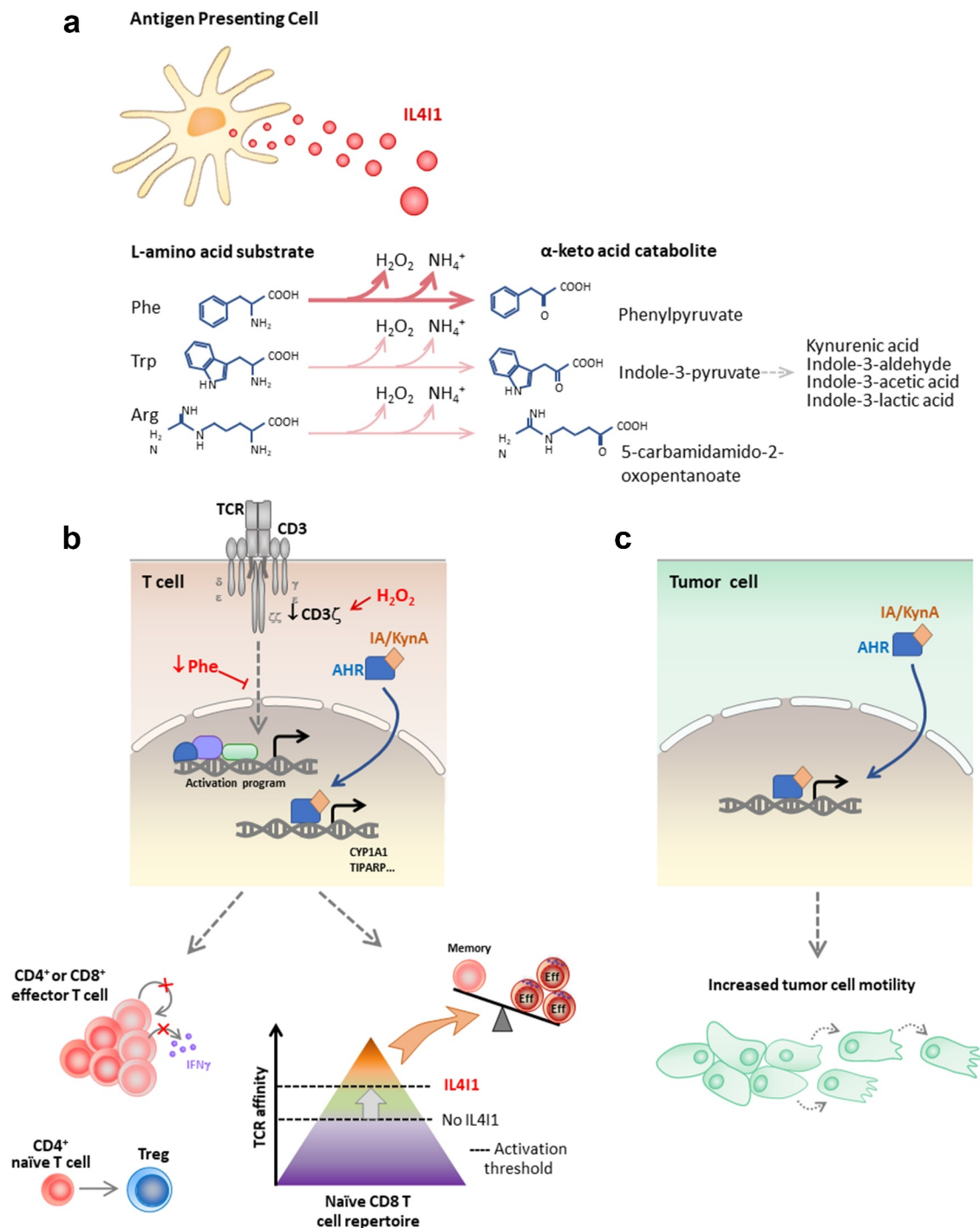


Figure 4. Mechanism of action of IL411 in the context of cancer. (a) IL411 is secreted into the synaptic cleft between an antigen-presenting cell and a T cell. L-amino acid oxidation by IL411 produces an α -keto acid, while liberating hydrogen peroxide (H_2O_2) and ammonia (NH_4^+). Phe is the most rapidly degraded amino acid but Trp and arginine (Arg) are also IL411 substrates. Sadik *et al.* showed that indole-3-pyruvate produced by Trp degradation is not detectable, as it is rapidly converted into non AHR-stimulating indole derivatives and kynurenic acid (KynA) and indole-3-aldehyde (IA), which are AHR agonists. (b) IL411 enzymatic activity is responsible for the consumption of Phe, which deprives T cells of this essential amino acid, and potentially participates in limiting the availability of Trp and Arg. This leads to decreased activation of the mTORC1 pathway downstream of the TCR and costimulatory receptors. T-cell signaling is also sensitive to hydrogen peroxide, which reduces membrane exposure of the CD3 ζ chain. The mechanism of inhibition of early TCR signaling (ZAP70 activation) is still unresolved. Phe depletion and IL411-produced second messengers limit effector T-cell proliferation and favor regulatory T-cell differentiation from naïve CD4 T cells, individually or in combination. As shown by Puiffe *et al.*, IL411 also increases the threshold of CD8 T-cell activation, thus restraining the primed repertoire to high-affinity clones and favoring the differentiation of effector cells at the expense of memory cells. Cancer development is favored by the diminution of the quality and intensity of the T-cell response. (c) Metastasis may also be promoted by increased tumor-cell motility induced by KynA and IA stimulation of the AHR pathway in tumor cells.

Overall, it has been clearly demonstrated in the literature that IL411 alters the antitumor CD8 T-cell response and that IL411-mediated immunoregulation facilitates cancer growth, affecting patient survival and potentially fostering resistance to immune

checkpoint inhibitors. Despite these new advances, there are still uncertainties concerning the mechanism of the action of IL411 on the T-cell response at the molecular and cellular levels. Recent data suggest that IL411 affects directly both CD8 T cells

and tumor cells (Figure 4). Indeed, IL4I1-dependent modulation of the affinity and functional properties of the CD8 T-cell repertoire elicited at priming may interfere with its capacity to combat tumor cells.²³ In this case, IL4I1 effects on early T cell signaling and immune synapse formation are too rapid to involve gene regulation. In particular contexts where sufficient Trp catabolites are generated, IL4I1-mediated AHR activation may play a role in enhancing tumor-cell migration and metastasis,¹¹ or tumor cell survival, as I3P has also been recently proposed to elicit a cell protective gene expression program.³² Pharmacological blockade of IL4I1 is therefore of high interest.

In contrast to other immunoregulatory enzymes, IL4I1 is a secreted protein and is therefore easily accessible to pharmacological blockade.³³ An orally-available compound with potent IL4I1 inhibitory activity *in vitro* has been recently developed.³¹ This compound limits the growth of several tumors in mouse models, without significant toxicity, opening the way to IL4I1 targeting in human cancer. Thus, a complete understanding of the actions of IL4I1 will be fundamental to facilitating clinical development and avoiding the failure that occurred with epacadostat.¹²

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

- Murray PJ. Amino acid auxotrophy as a system of immunological control nodes. *Nat Immunol.* 2016;17:132–139. doi:10.1038/ni.3323.
- Van Den Eynde BJ, Van Baren N, Baurain J-F. Is There a clinical future for IDO1 inhibitors after the failure of epacadostat in melanoma? *Annu Rev Cancer Biol.* 2020;4:241–256. doi:10.1146/annurev-cancerbio-030419-033635.
- Boulland M-L, Marquet J, Molinier-Frenkel V, Möller P, Guiter C, Lasoudris F, Copie-Bergman C, Baia M, Gaulard P, Leroy K, et al. Human IL4I1 is a secreted L-phenylalanine oxidase expressed by mature dendritic cells that inhibits T-lymphocyte proliferation. *Blood.* 2007;110:220–227. doi:10.1182/blood-2006-07-036210.
- Marquet J, Lasoudris F, Cousin C, Puiffe M-L, Martin-Garcia N, Baud V, Chereau F, Farcet J-P, Molinier-Frenkel V, Castellano F. Dichotomy between factors inducing the immunosuppressive enzyme IL-4-induced gene 1 (IL4I1) in B lymphocytes and mononuclear phagocytes. *Eur J Immunol.* 2010;40:2557–2568. doi:10.1002/eji.201040428.
- Cousin C, Aubatin A, Le Gouvello S, Apetoh L, Castellano F, Molinier-Frenkel V. The immunosuppressive enzyme IL4I1 promotes FoxP3(+) regulatory T lymphocyte differentiation. *Eur J Immunol.* 2015;45:1772–1782. doi:10.1002/eji.201445000.
- Carbonnelle-Puscian A, Copie-Bergman C, Baia M, Martin-Garcia N, Allory Y, Haioun C, Crémades A, Abd-Asamad I, Farcet J-P, Gaulard P, et al. The novel immunosuppressive enzyme IL4I1 is expressed by neoplastic cells of several B-cell lymphomas and by tumor-associated macrophages. *Leukemia.* 2009;23:952–960. doi:10.1038/leu.2008.380.
- Maier B, Leader AM, Chen ST, Tung N, Chang C, LeBerichel J, Chudnovskiy A, Maskey S, Walker L, Finnigan JP, et al. A conserved dendritic-cell regulatory program limits antitumor immunity. *Nature.* 2020;580:257–262. doi:10.1038/s41586-020-2134-y.
- Liu Y, He S, Wang X-L, Peng W, Chen Q-Y, Chi D-M, Chen J-R, Han B-W, Lin G-W, Li Y-Q, et al. Tumour heterogeneity and intercellular networks of nasopharyngeal carcinoma at single cell resolution. *Nat Commun.* 2021;12(741).
- Lasoudris F, Cousin C, Prevost-Blondel A, Martin-Garcia N, Abd-Asamad I, Ortonne N, Farcet J-P, Castellano F, Molinier-Frenkel V. IL4I1: an inhibitor of the CD8+ antitumor T-cell response *in vivo*. *Eur J Immunol.* 2011;41:1629–1638. doi:10.1002/eji.201041119.
- Bod L, Lengagne R, Wrobel L, Ramspott JP, Kato M, Avril M-F, Castellano F, Molinier-Frenkel V, Prevost-Blondel A. IL4-induced gene 1 promotes tumor growth by shaping the immune microenvironment in melanoma. *Oncoimmunology.* 2017;6(e1278331): e1278331. doi:10.1080/2162402X.2016.1278331.
- Sadik A, Somarribas Patterson LF, Öztürk S, Mohapatra SR, Panitz V, Secker PF, Pfänder P, Loth S, Salem H, Prentzell MT, et al. IL4I1 is a metabolic immune checkpoint that activates the AHR and promotes tumor progression. *Cell.* 2020;182(5):1252–1270. doi: 10.1016/j.cell.2020.07.038.
- Ramspott JP, Bekkat F, Bod L, Favier M, Terris B, Salomon A, Djerroudi L, Zaenker KS, Richard Y, Molinier-Frenkel V, et al. Emerging role of IL-4-induced gene 1 as a prognostic biomarker affecting the local T-cell response in human cutaneous melanoma. *J Invest Dermatol.* 2018;138:2625–2634. doi:10.1016/j.jid.2018.06.178.
- Liu M, Pan Q, Xiao R, Yu Y, Lu W, Wang L. A cluster of metabolism-related genes predict prognosis and progression of clear cell renal cell carcinoma. *Sci Rep.* 2020;10(1):12949. doi:10.1038/s41598-020-67760-6.
- Molinier-Frenkel V, Prevost-Blondel A, Castellano F. The IL4I1 enzyme: a new player in the immunosuppressive tumor microenvironment. *Cells.* 2019;8:757. doi:10.3390/cells8070757.
- Lee JH, Lee JE, Kahng JY, Kim SH, Park JS, Yoon SJ, Um J-Y, Kim WK, Lee J-K, Park J, et al. Human glioblastoma arises from subventricular zone cells with low-level driver mutations. *Nature.* 2018;560:243–247. doi:10.1038/s41586-018-0389-3.
- Wiemann S, Kolb-Kokocinski A, Poustka A. Alternative pre-mRNA processing regulates cell-type specific expression of the IL4I1 and NUP62 genes. *BMC Biol.* 2005;3(16):16. doi:10.1186/1741-7007-3-16.
- Gao Y, Mu J, Xu T, Linghu T, Zhao H, Tian J, Qin X. Metabolomic analysis of the hippocampus in a rat model of chronic mild unpredictable stress-induced depression based on a pathway crosstalk and network module approach. *J. Pharm. Biomed. Anal.* 2021;193(113755):113755. doi:10.1016/j.jpba.2020.113755.
- Castellano F, Molinier-Frenkel V. Control of T-cell activation and signaling by amino-acid catabolizing enzymes. *Front Cell Dev Biol.* 2020;8:613416. doi:10.3389/fcell.2020.613416.
- Paris A, Tardif N, Galibert M-D, Corre S. AHR and cancer: from gene profiling to targeted therapy. *Int. J. Mol. Sci.* 2021;22(752):752. doi:10.3390/ijms22020752.
- Rouillard AD, Gunderson GW, Fernandez NF, Wang Z, Monteiro CD, McDermott MG, Ma'ayan A. The harmonizome:

- a collection of processed datasets gathered to serve and mine knowledge about genes and proteins. Database. 2016;2016 (baw100):baw100. doi:10.1093/database/baw100.
21. Lo R, Matthews J. High-resolution genome-wide mapping of AHR and ARNT binding sites by ChIP-Seq. *Toxicol Sci Off J Soc Toxicol.* 2012;130:349–361. doi:10.1093/toxsci/kfs253.
 22. Oshchepkova E, Sizentsova Y, Wiebe D, Mironova V, Kolchanov N. Meta-analysis of transcriptome data detected new potential players in response to dioxin exposure in humans. *Int J Mol Sci.* 2020;21:7858. doi:10.3390/ijms21217858.
 23. Puiffé M-L, Dupont A, Sako N, Gatineau J, Cohen JL, Mestivier D, Lebon A, Prévost-Blondel A, Castellano F, Molinier-Frenkel V. IL4I1 accelerates the expansion of effector CD8+ T cells at the expense of memory precursors by increasing the threshold of T-cell activation. *Front Immunol.* 2020;11(600012). doi:10.3389/fimmu.2020.600012.
 24. Aubatin A, Sako N, Decrouy X, Donnadiou E, Molinier-Frenkel V, Castellano F. IL4-induced gene 1 is secreted at the immune synapse and modulates TCR activation independently of its enzymatic activity. *Eur J Immunol.* 2018;48:106–119. doi:10.1002/eji.201646769.
 25. Riaz N, Havel JJ, Makarov V, Desrichard A, Urba WJ, Sims JS, Hodi FS, Martín-Algarra S, Mandal R, Sharfman WH, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. *Cell.* 2017;171:934–949.e16. doi:10.1016/j.cell.2017.09.028.
 26. Goldman MJ, Craft B, Hastie M, Repečka K, McDade F, Kamath A, Banerjee A, Luo Y, Rogers D, Brooks AN, et al. Visualizing and interpreting cancer genomics data via the Xena platform. *Nat Biotechnol.* 2020;38:675–678. doi:10.1038/s41587-020-0546-8.
 27. Zhang X, Gan M, Li J, Li H, Su M, Tan D, Wang S, Jia M, Zhang L, Chen G. Endogenous Indole Pyruvate Pathway for Tryptophan Metabolism Mediated by IL4I1. *J Agric Food Chem.* in press. 2020;68(39):10678–10684. doi:10.1021/acs.jafc.0c03735.
 28. Zhai L, Ladomersky E, Lenzen A, Nguyen B, Patel R, Lauring KL, Wu M, Wainwright DA. IDO1 in cancer: a Gemini of immune checkpoints. *Cell Mol Immunol.* 2018;15:447–457. doi:10.1038/cmi.2017.143.
 29. Fong MY, McDunn J, Kakar SS. Identification of metabolites in the normal ovary and their transformation in primary and metastatic ovarian cancer. *PLoS One.* 2011;6(e19963). doi:10.1371/journal.pone.0019963.
 30. Soliman GA, Steenson SM, Eteko AH. Effects of metformin and a mammalian target of rapamycin (mTOR) ATP-competitive inhibitor on targeted metabolomics in pancreatic cancer cell line. *Metabolomics Open Access.* 2016;6. doi:10.4172/2153-0769.1000183.
 31. MacKinnon A, Bhupathi D, Chen J, Huang T, Li W, Ma Y, Sotirovska N, Steggerda S, Zhang W, Parlati F. 705 Anti-tumor activity of CB-668, a potent, selective and orally bioavailable small-molecule inhibitor of the immuno-suppressive enzyme interleukin 4 (IL-4)-induced gene 1 (IL4I1). *J Immunother Cancer.* 2020;8:A747–A747. doi:10.1136/jitc-2020-SITC2020.0705.
 32. Zeitler L, Fiore A, Meyer C, Russier M, Zanella G, Suppmann S, Gargaro M, Sidhu SS, Seshagiri S, Ohnmacht C, et al. Anti-ferroptotic mechanism of IL4i1-mediated amino acid metabolism. *eLife.* 2021;10. doi:10.7554/eLife.64806.
 33. Pisset M, Djordjevic D, Dupont A, Le Gall E, Molinier-Frenkel V, Castellano F. Identification of inhibitors of the immunosuppressive enzyme IL4I1. *Bioorganic Chem.* 2020;94(103463):103463. doi:10.1016/j.bioorg.2019.103463.